Gaining Quantitative Concentration Information from a Two-Dimensional Projection of a Three-Dimensional Sample

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X-ray fluorescence microprobe beamlines have provided a unique method for the determination of spatial and temporal concentration and spectroscopic changes in the zinc environment of zebrafish embryos during early development. The X-ray fluorescence microprobe is the only technique that can provide element specific information on intact biological samples. However, the embryos are ca. 700 μm in diameter, and therefore the resulting two-dimensional images have to be corrected for the volume differences across the embryo and the X-ray attenuation of the incident and fluorescence intensity as it passes through the cell matrix. Since the corrections are going to ultimately be applied to a spherical zebrafish embryo, which is >70% water with Zn concentrations of ca. 1 mM and trace amounts of other metal ions, the standards chosen were frozen bubbles of $ZnCl_2/FeCl_2$ solutions ranging in concentration from 0.5 mM – 2.5 mM.

Results

To accomplish the correction, images were measured of the standards. Since these standards are homogeneous solutions, the applied corrections should make any contrast in the image dissappear. Eq. 1-3 are the individual corrections that are applied to each row. Eq. 1 normalizes each pixel to the volume of the sample that is transversed by the beam. Eq. 2 corrects the measured I0 for the average thickness encountered by the

$$V = \left(2\sqrt{R^2 - (R-x)^2}\right) \bullet \left(4\mu m^2\right) \qquad (1)$$

$$I0_c = I0_m \bullet \left[\exp - \left(\left(4 \int_0^{diameter} \sqrt{R^2 - (R-x)^2}\, dx\right) \bullet A_{10KeV}\right)\right] \qquad (2)$$

$$FF_c = FF_m \bullet \left[\exp - \left((d-x) \bullet A_{8.6KeV}\right)\right] \qquad (3)$$

incident beam, while Eq. 3 corrects the fluorescence intensity for the thickness of the embryo between the excitation position and the detector. The attenuation factors used in Eq. 2 and 3 are found in the NIST data base for the total attenuation of water with coherent scattering[1].

Individual rows of the image were examined to determine the actual magnitude of the correction. Fig 1 shows the raw image of a 1 mM $ZnCl_2/FeCl_2$ bubble, the raw fluorescence counts normalized to the measured incident intensity from a single row, and the same row after the volume and intensity corrections have been applied. It is apparent from the image (left) and the uncorrected profile of the single row (upper right), that the side of the standard closest to the detector has a higher fluorescence intensity. However, after the corrections are applied, the profile of the single row (lower right) has a linear profile within the level of the noise, as would be expected for a homogeneous sample. The single row plots demonstrate that there is ca. 40% correction in the attenuation from

the water matrix. It has also been determined that at the concentrations in found in the zebrafish embryos, the metal ions do not add to the X-ray attenuation of the sample.

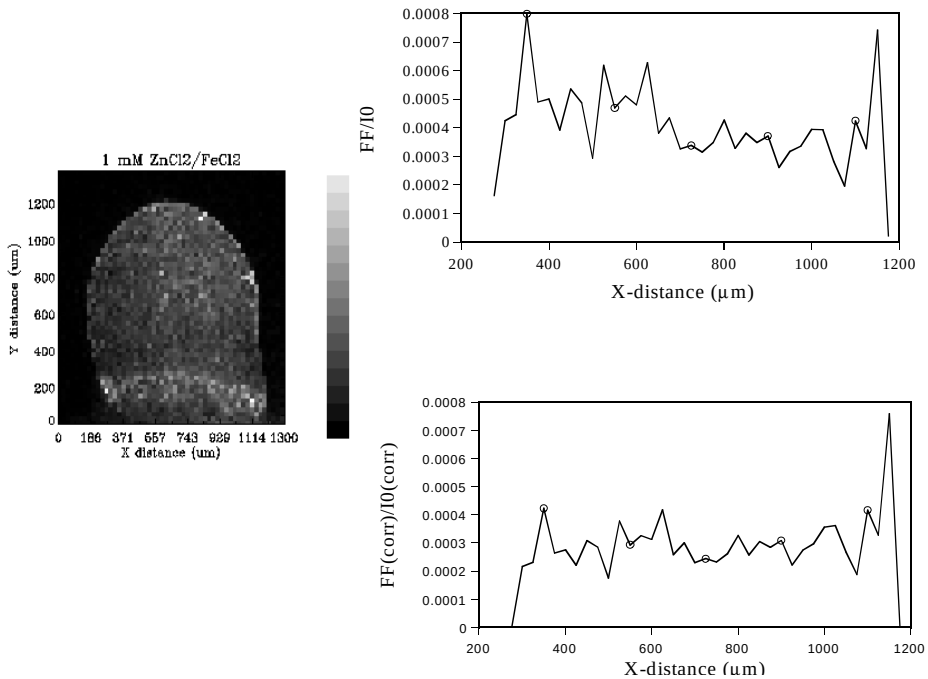

Figure 1. Image of a 1 mM $ZnCl_2/FeCl_2$ frozen solution (left), uncorrected fluorescence profile of a single row (top right), and the corrected fluorescence profile of the same row (lower right).

These corrections will be applied to similar measurements made on zebrafish embryos. The average fluorescence counts per pixel in the zebrafish embryos will be compared with those of the standards to determine for the first time the in situ Zn flux from the yolk to the dividing blastomeres at these earliest stages of development.

[1] Physical reference data can be found at www.physics.nist.gov.

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